CANADA COMMUNICABLE DISEASE REPORT

CCDR

May 29, 2014 • Volume 40•11
ISSN 1481–8531

Inside this issue: Clinical aspects of Lyme disease

As summer approaches, consider this: Lyme disease is expanding its reach in Canada, the United States (US), Europe and Asia. A previous issue identified where Lyme disease occurs in Canada. In this issue, read about how Lyme disease presents, what is needed to make the diagnosis, current treatment recommendations and best practices for laboratory testing. See links below to find out where Lyme disease is most common in the US and abroad, and learn the four key prevention messages. Bring this to the attention of those who are planning to camp, hike or spend time in the woods.

Review articles

Lyme disease: clinical diagnosis and treatment 194
Hatchette TF, Davis I and Johnston BL

Laboratory diagnostics for Lyme disease 209
Lindsay LR, Bernat K and Dibernardo A

Useful links

Public Health Notice: Lyme disease. Public Health Agency of Canada
http://www.phac-aspc.gc.ca/phn-asp/2013/lyme-0730-eng.php

See this overview with information on risk to Canadians, how to prevent Lyme disease and many useful resources, such as Frequently Asked Questions and provincial websites.

International Travel and Health: Lyme borreliosis (Lyme disease). World Health Organization
http://www.who.int/ith/diseases/lyme/en/

There are foci of Lyme borreliosis in forested areas of Asia, north-western, central and eastern Europe, and the US.

Lyme disease data. United States (US) Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/lyme/stats/index.html

In 2012, 95% of Lyme disease cases were reported from 13 states: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Virginia and Wisconsin.

Lyme disease prevention messages. US CDC
http://www.cdc.gov/lyme/

Wear repellent. Check for ticks daily. Shower soon after being outdoors. Call your doctor if you get a fever or rash.

Next issue June 12, 2014: Measles

Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Lyme disease: clinical diagnosis and treatment

Hatchette TF[1,2,3*], Davis I[1,2] and Johnston BL[2]

[1] Department of Pathology and Laboratory Medicine, Capital District Health Authority (CDHA), Halifax, Nova Scotia
[2] Department of Medicine, CDHA and Dalhousie University, Halifax, Nova Scotia
[3] Department of Pathology, Dalhousie University, Halifax, Nova Scotia
* Corresponding author: todd.hatchette@cdha.nshealth.ca

Abstract

Background: Lyme disease is an emerging zoonotic infection in Canada. As the *Ixodes* tick expands its range, more Canadians will be exposed to *Borrelia burgdorferi*, the bacterium that causes Lyme disease.

Objective: To review the clinical diagnosis and treatment of Lyme disease for front-line clinicians.

Methods: A literature search using PubMed and restricted to articles published in English between 1977 and 2014.

Results: Individuals in Lyme-endemic areas are at greatest risk, but not all tick bites transmit Lyme disease. The diagnosis is predominantly clinical. Patients with Lyme disease may present with early disease that is characterized by a "bull's eye rash", fever and myalgias or with early disseminated disease that can manifest with arthralgias, cardiac conduction abnormalities or neurologic symptoms. Late Lyme disease in North America typically manifests with oligoarticular arthritis but can present with a subacute encephalopathy. Antibiotic treatment is effective against Lyme disease and works best when given early in the infection. Prophylaxis with doxycyline may be indicated in certain circumstances. While a minority of patients may have persistent symptoms, evidence does not demonstrate that prolonged courses of antibiotics improve outcome.

Conclusion: Clinicians need to be aware of the signs and symptoms of Lyme disease. Knowing the regions where *Borrelia* infection is endemic in North America is important for recognizing patients at risk and informing the need for treatment.

Introduction

Lyme disease is the most common vector-borne zoonosis in North America, where it is estimated that each year more than 300,000 individuals in the United States (US) are infected by *Borrelia burgdorferi*, the bacterium that causes Lyme disease(1). Data from Canada are sparse, as Lyme disease only became nationally reportable in 2009. The number of reported infections in Canada in 2012 was 315, which is likely an underestimate and includes cases that are the result of infections acquired during travel to other endemic regions, such as Europe (2-4).

The range of *Ixodes* ticks, the vector for *Borrelia*, has expanded greatly over the last 20 years, making Lyme disease an emerging infection in Canada. *Ixodes* ticks have been identified in British Columbia, southeastern Manitoba, southern and eastern Ontario, and specific areas in New Brunswick, Nova Scotia and Quebec (4,5). As the *Ixodes* tick expands its range, more Canadians will be exposed to *B. burgdorferi*, and clinicians will need the expertise to manage tick bites and diagnose and treat Lyme disease.

Awareness of the extent of local Lyme disease, early detection and treatment are important for managing this emerging infection. The objective of this article is to review the clinical diagnosis and treatment of *B. burgdorferi* infection for the front-line clinician.

Methods

A literature search was done using PubMed and restricted to articles published in English between 1977 and 2014. The following search terms were used: Lyme disease and treatment, Lyme disease and arthritis, Lyme disease and clinical presentation , Lyme disease ticks and risks, Lyme disease and pregnancy, neuroborreliosis, chronic Lyme OR post-Lyme syndrome AND treatment (clinical trials). Abstracts were reviewed for clinically relevant articles, and additional references were obtained from the articles identified in the initial search. Although there are different strains of *Borrelia* in North America and Europe, which are associated with overlapping but distinct clinical presentations, this review will focus on the clinical manifestations that are common to Canada. It does not describe the various aspects of diagnostic testing, which is reviewed in a companion paper (6).

Findings

Who is at risk of Lyme disease and why?

An individual must first be bitten by a tick that is infected with *B. burgdorferi* for infection to occur. While infected ticks can be sporadically deposited in different regions in Canada by migratory birds, the risk of getting Lyme disease is greatest in an area where the ticks are persistently present or endemic, and the risk depends on the proportion of ticks that are infected. Even in areas where the ticks have become endemic, the proportion infected by this spirochete varies and is evolving. Clinicians may want to check with their local public health departments to understand where the current Lyme endemic areas are within their jurisdiction. It is important to obtain a thorough travel history from the patient, as Lyme disease is endemic in other areas of North America and Europe, and travel-related Lyme disease accounts for a proportion of the documented cases in Canada. It is also important to relay this travel history or suspicion of European *Borrelia* infection to the diagnostic laboratory, as the confirmatory testing for North American and European *Borrelia* strains differs.

What is the risk of Lyme after a tick bite?

While studies are limited in numbers and size, data from the US and Europe suggest that the risk of acquiring Lyme disease, even after a bite from an infected tick, is small – in the range of < 1% to6% (7). In studies that have examined the efficacy of antibiotic prophylaxis in persons presenting with a tick bite in Lyme-endemic areas (where the prevalence of *B. burgdorferi* in ticks ranged from 15% to 50%), the rate of infection among those who received placebo ranged from 1.1% to 3.4% (8–11). These rates are consistent with a prospective study of patients bitten by ticks in a Lyme-endemic area of New York State, where infection developed in 3.7% of those bitten by a tick (12).

The risk of infection following a tick bite is related to how long the tick has been attached and is explained by the *Borrelia* life cycle in the tick. The spirochete first has to migrate from the tick's gut to its salivary glands before it can be injected into the human through the bite. In order to do this, the bacterium must undergo antigenic change to its outer coat that favours human infection (13–16). This process takes about 36 hours (14). If the tick is removed during this time frame, infection is almost always prevented. In an antibiotic prophylaxis trial in the US, no study participants receiving placebo or prophylaxis were infected if the tick fed for less than 72 hours (11). This is further supported by data from another prospective study in an endemic region, in which participants were at greatest risk of infection if the tick had been attached for more than 72 hours (12).

Who should receive prophylaxis after a tick bite?

There are a number of factors that require consideration when deciding whether to offer prophylaxis after a tick bite (**Table 1**). All of the prophylaxis trials have been conducted in regions that would be considered hyperendemic for Lyme disease, where the burden of infection in ticks is > 20% and where the antibiotics were administered within 72 hours of removing the tick (8–11). An early meta-analysis of antibiotics for prophylaxis of Lyme disease reported a pooled infection rate in those who received placebo of 1.4%, compared with 0% for those receiving an antibiotic (17). Each of the included trials was small and failed to show any significant effect of prophylaxis, as the incidence of infection in those taking placebo was low (8-10). A subsequent randomized placebo controlled trial showed that a single dose of doxycycline could reduce the rate of Lyme disease (11).

Table 1. Criteria and recommended prophylaxis after a tick bite (19)

Criteria for prophylaxis*	1. The attached tick can be reliably identified as an *I. scapularis* (deer/blacklegged) tick that is estimated to have been attached for > 36 h on the basis of the degree of engorgement or by certainty about the time of tick acquisition.	
	2. Prophylaxis can be started within 72 h of tick removal.	
	3. The local rate of *B. burgdorferi* infection in ticks is > 20% (check with local public health).	
	4. Doxycycline is not contraindicated.	
Recommended prophylaxis	Adults and children > 8 years of age	Single 200 mg dose of doxycycline given orally (4.4 mg/kg for patients < 45 kg)
	Children < 8 years of age and pregnancy	Not recommended

* Consider antibiotic prophylaxis after a tick bite when the individual meets all of the criteria.

An updated systematic review and meta-analysis suggested that, to prevent one case of Lyme disease, prophylaxis of 49 cases would be needed (18). Patients in whom infection developed were successfully treated without any long-term sequelae. Given that there were relatively few infections in each of the studies and that these were successfully treated, the use of antimicrobial prophylaxis must be considered in the context of potential side effects, which were reported but were not severe.

How do patients with Lyme disease present and how are they treated?

Although patients with *B burgdorferi* infection can be asymptomatic, most cases of Lyme disease present as one of three stages, which may occur sequentially if an earlier stage was untreated: early localized disease (usually < 30 days from exposure), early disseminated disease (< 3 months after exposure) and late disseminated disease (> 3 months after exposure) (**Table 2**).

Table 2. Clinical manifestations of Lyme disease (19,24,39)

Stage	System	Manifestation
Early localized disease (< 30 days)*	Skin	Erythema migrans (note: must be > 5 cm in diameter, painless and slowly expanding)
	Systemic	Fever
		Arthralgias
		Headache
Early disseminated disease (< 3 months)*	Skin	Multiple erythema migrans
	Systemic	Fever
		Arthralgias
		Headache
		Lymphadenophathy
	Heart	Atrioventricular block
		Tachyarrhythmias
		Myopericarditis
		Myocardial dysfunction
	CNS	Aseptic meningitis
		Cranial neuropathy (especially facial nerve palsy)
		Motor or sensory radiculopathy

Stage	System	Manifestation
	Ocular	Conjunctivitis (rare)
Late disseminated disease (> 3 months)*	MSK	Oligoarticular arthritis
	CNS	Encephalopathy
		Axonal polyradiculoneuropathy
		Chronic encephalomyelitis
	Ocular	Retinitis (rare)

* Note: While there may be exceptions, these time frames provide clinicians with a general guide on when the different manifestations tend to occur.
CNS = central nervous system.
MSK = musculoskeletal.

The treatment of Lyme disease depends on the stage and organ system involved and is summarized in a clinical practice guideline that has been developed by the Infectious Diseases Society of America (IDSA) **(Table 3)** (19). The clinical presentation and some of the literature that supports those treatment recommendations are summarized in the sections that follow.

Table3. Infectious Disease Society of America guidelines for the treatment of Lyme disease (19)*

Treatment of adults and children older than 8 years with Lyme disease	Erythema migrans or early disseminated disease, including Bell's palsy, but **without** other CNS involvement	• Doxycycline 100 mg po bid x 14–21 days (contraindicated in pregnancy) • Amoxicillin 500 mg po tid x 14–21 days • Cefuroxime 500 mg po bid x 14–21 days
	Early Lyme **with** CNS involvement	• Ceftriaxone 2 g IV once daily x 14–28 days • Pen G 4 x10^6 units IV q 4 h x 14–28 days • Doxycycline 100–200 mg po bid x 28 days (alternative if others not possible)
	Early Lyme **with** carditis	• Same treatment as early Lyme with CNS involvement, but use IV initially with high grade heart block or if admission to hospital is necessary.
	Late Lyme **without** CNS involvement**	• Doxycycline 100 mg po bid x 28 days • Amoxicillin 500 mg po tid x 28 days • Cefuroxime 500 mg po bid x 28 days
	Late Lyme **with** CNS involvement (late neuroborreliosis)	• Ceftriaxone 2 g IV once daily x 14–28 days • Pen G 4 x10^6 units IV q 4 h x 14–28 days
Treatment of children 8 years or younger with Lyme disease	Early localized disease	• Amoxicillin 30 mg/kg per day, orally, divided into three doses (max 1.5 g/day) for 14–21 days • For children allergic to penicillin, cefuroxime 30 mg/kg per day, orally, in two divided doses (maximum 1 g/day) for 14–21 days
	Early disseminated and late disease: *multiple erythema migrans*	• Oral treatment as the above for 21 days
	Early disseminated and late disease:	• Oral treatment as the above for 21 days

	isolated facial palsy and first episodes of arthritis	
	Early disseminated and late disease: *persistent/recurrent arthritis, carditis and meningitis/encephalitis*	• Ceftriaxone or penicillin IV at pediatric dosing

* These recommendations are off-label, evidence-based best practices.
** Recurrent or persistent joint swelling – repeat 4 week course of oral antibiotic as above. Use of IV ceftriaxone should be reserved for relapse or persistent joint swelling without improvement with oral treatment.
CNS = central nervous system.

Asymptomatic infection

Approximately 1.6%–7% of infected individuals may have asymptomatic infection (7, 20). The prognosis for patients with asymptomatic infection is generally good. Within a large vaccine study conducted in 10 US states where Lyme is endemic, asymptomatic infections were documented in 6% (15/269) of study participants. While the majority of study participants received treatment when the seroconversion had been documented, only 1 of 8 who did not receive treatment went on to show arthritis over the 12 months after seroconversion (20). Although the follow-up period was relatively short, these data support the notion that asymptomatic infection does not require treatment, and disease will ultimately declare itself if infection persists.

Early localized disease (< 30 days)

Early disease usually presents as an acute illness characterized by fever, headache and myalgia with the presence of a single, localized skin lesion known as erythema migrans (EM). This characteristic skin eruption is present in approximately 80% of patients with early disease and will resolve without antibiotic treatment over a median of 28 days (21, 22). While most patients will present with erythema migrans within seven days of the initial tick bite, the incubation period can vary between 3 and 30 days (23). The skin lesion is characteristically an annular erythematous lesion > 5 cm in diameter that slowly increases in size and is usually painless and non-pruritic. The lesion sometimes develops central clearing, but it can be more homogenously erythematous (19, 23, 24). It is important to note that many people can have a reaction to the tick saliva and show a localized reaction resembling erythema migrans. However, unlike erythema migrans this reaction often develops within the first three days of the bite, is 5 cm or less in diameter and does not expand.

Erythema migrans can be subtle or even go unnoticed, particularly if the bite was in an area that is difficult for the patient to see, such as behind the knee. Diagnostic testing is insensitive at this stage and not recommended in a patient who has links to an endemic area (6).

Treatment: Several clinical trials conducted in the US (23, 25–28) and Europe (29–31) has informed the antimicrobial treatment of early Lyme disease. In these trials, individuals with a clinical diagnosis of erythema migrans were randomly assigned to one of several treatment options. The agents used included amoxicillin, penicillin, ceftriaxone, a macrolide (erythromycin or azithromycin) and a tetracycline or doxycycline in somewhat varying doses and for varying lengths of times. Azithromycin therapy was given for 5 or 10 days, beta-lactams were given for 10–30 days, and tetracycline/doxycycline was given for 10, 14 or 20–21 days. Follow-up frequently lasted for several months after completion of therapy, and patients were monitored for complete and partial resolution of symptoms, treatment failure and late manifestations of illness that were considered either minor or major.

The studies found similar efficacy for amoxicillin, azithromycin, cefuroxime and doxycycline independent of the dose and duration of therapy. The one trial that included erythromycin showed it to be less effective than penicillin and tetracycline. A large retrospective cohort study of 607 patients confirmed these findings, 99% of patients with early local or disseminated Lyme remaining treatment-failure free at two years whether treated for ≤ 10 days, 11–15 days or > 15 days (32). One study that looked specifically at early disseminated Lyme disease compared ceftriaxone 2 g daily for 2 weeks with doxycycline 100 mg twice daily for 3 weeks (33). Clinical cure was similar for the two (85% for ceftriaxone and 88% for the doxycycline), with 14% (doxycycline) and 27%

(ceftriaxone) of patients having residual symptoms, usually mild arthralgias and fatigue, at nine months after completing treatment.

Prognosis: Both clinical trials (23, 25–31) and observational studies (32,34–36) provide information about the prognosis for treated early Lyme disease. With the currently recommended therapies, greater than 80% of patients will have complete resolution of symptoms at long-term follow-up. While it is not unusual for individuals to have symptoms such as fatigue and arthralgias after treatment for Lyme, most studies reported either no or few (< 5%) late manifestations of disseminated Lyme.

To determine whether patients who had early Lyme disease are more likely to have persistent symptoms after their acute infection, investigators in Slovenia compared a cohort of patients treated for erythema migrans with a control group of individuals who did not have Lyme disease (36). They found that the frequency of new or increased symptoms in patients with this condition did not exceed the frequency of such symptoms in the control group. At 12 months, only 2.2% of Lyme disease patients reported new or increased symptoms, and in none of the patients were the symptoms disabling. These findings are similar to those of a study done in the US involving patients with and without Lyme disease from 1984 to 1991 (37). The frequency of new symptoms and increased difficulties with daily activities were similar in the two groups, those with Lyme disease and age-matched controls without Lyme disease.

In summary, the evidence from these studies suggests that although patients with Lyme may have symptoms that persist or appear after treatment is complete, the frequency of those symptoms is comparable with what one would see in individuals who do not have Lyme disease.

Early disseminated Lyme disease (< 3 months)

Early disease may be followed by disseminated disease, with the development of multiple secondary annular lesions and multisystem and intermittent cardiac, neurological, ocular or articular manifestations. The development of multiple secondary annular lesions and systemic symptoms, including fever, arthralgias, headache and lymphadenopathy, usually occurs within several weeks of the localized erythema migrans (38). In one early study, half of the patients presenting with this condition went on to show multiple erythema migrans lesions (38).

The other manifestations of early disseminated infection, such as Lyme carditis and neurologic infection (known as neuroborreliosis), tend to occur weeks to months after initial infection (38). Patients with Lyme carditis can present with conduction abnormalities, tachyarrhythmias, myopericarditis or myocardial dysfunction (39). The most common presentation is atrioventricular block ranging from first to third degree heart block, often requiring temporary pacing. Carditis usual occurs within two months of the presenting erythema migrans, but a history of the condition is not always present.

Treatment is effective, and carditis is rarely fatal (40). While earlier data showed that carditis developed in up to 10% of patients with untreated Lyme (39), recent data suggest that rates are much lower; this may be due to improved recognition of *Borrelia* infection and effective treatment (19). US data from 1996 to 2006 suggest that only 0.8% of patients reported to have Lyme disease have conduction abnormalities (41). Physicians should think of Lyme as a cause of unexplained conduction abnormalities in patients who have recently been to a Lyme-endemic area. However, in the absence of a history of erythema migrans, the presentation is too non-specific to warrant empiric treatment without serological confirmation (19).

Neuroborreliosis has a clinical spectrum that can include meningitis, facial paralysis, motor or sensory radiculopathy, and cognitive symptoms. In North America, neuroborreliosis commonly presents with cranial nerve involvement (especially Bell's palsy), with or without aseptic meningitis. Symptoms can appear anywhere from two to eight weeks after erythema migrans (42). Lyme disease can be responsible for Bell's palsy in 10%–50% of children and adults in Lyme-endemic areas and can be bilateral in up to 23% of patients (43–47). Clinicians should suspect Lyme disease as the cause of facial palsy when it occurs in Lyme-endemic areas during the tick season, is associated with headache and other non-cranial nerve neurologic findings, including papilledema, or is bilateral (44, 47–50).

In regions where Lyme is endemic, it can be challenging to differentiate early neuroborreliosis from other causes of central nervous system infection. The cerebrospinal fluid is usually abnormal, with a white cell count that is

elevated to levels similar to those of patients presenting with viral meningitis (48, 51). However, compared with other causes of aseptic meningitis, patients with Lyme meningitis are less likely to have fever and more likely to have a higher preponderance of mononuclear cells (total percentage of monocytes and lymphocytes) in the cerebrospinal fluid, a longer duration of headache, and cranial nerve involvement (48,52,53).

The rule of sevens: A clinical prediction rule has been developed to help stratify the likelihood of Lyme-related meningitis in children. Children are unlikely to have Lyme-related meningitis if they have all of the following: headache for fewer than seven days, less than 70% mononuclear cells in their cerebrospinal fluid and absence of a seventh nerve palsy. This "rule of 7s" has 96% sensitivity and 95% specificity for distinguishing aseptic meningitis from Lyme neuroborreliosis. On the basis of the rule of 7s, patients who lack these features can be managed conservatively while Lyme serological results are pending (53, 54).

Treatment: A study done in the US in the early 1990s of patients with disseminated Lyme disease(without meningitis) compared ceftriaxone 2 g daily for 14 days with doxycycline 100 mg twice daily for 21 days (33). The cure rate was similar for the two regimens (88% for ceftriaxone and 85% for doxycycline), 27% of ceftriaxone-treated patients and 14% of doxycycline- treated patients having residual symptoms at the end of treatment.

In a large series of 101 adults with Lyme-related Bell's palsy, the palsy resolved completely in more than 86% at a median time of 26 days (range 1–270 days) despite the fact that only 36% had received antibiotic treatment. Fifteen percent had mild residual weakness, and only one person had a severe deficit. Patients with residual dysfunction were more likely to have bilateral disease (43).

The prognosis in children is equally good. In a cohort study, 94% of children treated with antibiotics were asymptomatic four weeks after treatment. All but one patient had resolution of symptoms by six months, and no children had evidence of chronic disease two years later (34). In 43 children who presented with Bell's palsy attributable to Lyme disease, most felt that they were cured, and there was no difference in the daily activities of infected children compared with those of a non-infected group (55). These studies in patients with Lyme neuroborreliosis highlight the importance of early recognition and treatment in order to improve response to treatment. At the same time, it was demonstrated that a prolonged course of amoxicillin given orally after a three week course of intravenous ceftriaxone did not alter outcome as compared with placebo (56).

Late Lyme disease (> 3 months)

If left untreated, Lyme disease can evolve into late disease, which presents with persistent arthritis and/or persistent neuroborreliosis. The different strains of *Borrelia* in North America and Europe are associated with overlapping but distinct clinical presentations. In Europe, late Lyme disease presents with dermatologic syndromes that are rare in North America, including acrodermatitis chronica atrophicans and lymphocytoma cutis (24). In North America, arthritis is a much more common manifestation of late Lyme disease, whereas in Europe neuroborrelia is more commonly seen and presents as a chronic encephalitis or painful radiculopathy called Bannwarth's syndrome in up to 86% of cases (57).

If European Lyme disease is suspected on the basis of the clinical presentation and the patient's travel history, the laboratory should be notified to ensure that the appropriate serological test can be used, as the confirmation tests for North American and European *Borrelia* are different. A more detailed comparison of the differences between North American and European Lyme disease has been provided by Hengge and colleagues (24).

Late Lyme arthritis

In North America, up to 60% of untreated Lyme disease cases show monoarticular or oligoarticular arthritis, usually involving the knees, with other joints such as the ankle, elbow and wrist less commonly affected (58–60). In one study, all patients with late Lyme arthritis had symptoms appear within two years of infection. In 14 of 16 patients with untreated Lyme-related facial nerve palsies arthritis ultimately developed (61). Remembering a tick bite has not been a helpful feature in diagnosing Lyme arthritis, as up to 84% of cases did not recall exposure to a tick (32, 62).

Lyme disease serology is very sensitive and specific for the diagnosis of Lyme arthritis, and a negative test essentially rules out infection (6). However, the results of Lyme disease testing can take time, and because the presentation can mimic septic arthritis, which requires immediate treatment, it is important to try and differentiate the two.

Three studies have attempted to develop a clinical prediction model to distinguish Lyme arthritis from septic arthritis in Lyme-endemic areas. Although there is an overlap between the two entities, Lyme arthritis tends to involve the knees preferentially, is less frequently accompanied by fever and local signs of inflammation, and in general has lower inflammatory markers (62–64). One study found refusal to weight bear as the strongest predictor septic arthritis compared with Lyme disease (63). Another study in children found that an erythrocyte sedimentation rate of < 40 mm/hour and a peripheral white blood cell count < 10×10^3 cells/ mm^3 could effectively rule out septic arthritis (64). While the synovial fluid is inflammatory with elevated leukocyte counts, there is overlap with septic arthritis, and the cell count can range from $1,700 \times 10^3$ cells/µL to > $100,000 \times 10^3$ cells/µL, predominantly polymorphonuclear cells (58, 60, 63). In one review, children with Lyme disease had higher inflammatory markers than adults (60).

Up to 10% of patients will have evidence of synovitis six months after treatment for Lyme arthritis, termed antibiotic refractory Lyme arthritis (65). Currently, the only recommendation for the use of the polymerase chain reaction (PCR) to detect *Borrelia* DNA in the diagnosis of Lyme disease has been in patients with persistent synovial swelling, to determine whether retreatment is necessary (19). However, the significance of a positive test result after primary treatment is unclear. There are data to suggest that any *Borrelia* detected are not viable, calling into question whether there really is persistent infection in these patients (66).

Late Lyme arthritis treatment: There are few studies that have examined the treatment of late Lyme arthritis. Two studies (67,68) comparing penicillin or ceftriaxone with placebo in late Lyme arthritis in adults found benefit with antibiotic therapy, but the cure rate was still < 50%, although better with ceftriaxone than penicillin. A European randomized trial comparing penicillin and ceftriaxone, both intravenously for 10 days, in patients 13 years of age and older demonstrated a symptom remission rate of 87.9% for ceftriaxone and 61.3 % for penicillin. Finally, a study involving children and adults with late Lyme in the US in the mid-2000s (69) found that 28 days (70% cure) of ceftriaxone had no advantage over 14 days (76% cure) in terms of clinical cure and was associated with more treatment discontinuations due to adverse events. In some series up to 25% of children and 50% of adults required a second course of antibiotics because of refractory arthritis symptoms. Those who went on to show refractory arthritis were successfully treated with nonsteroidal anti-inflammatories, steroid injections or disease-modifying drugs. None developed long-term sequelae (59, 60).

Late neuroborreliosis

The most common manifestation of neuroborreliosis in North America is subacute encephalopathy with subtle cognitive changes, whereas a chronic encephalomyelitis characterized by spastic paraparesis and cognitive impairment is more common in Europe (24, 70). In one case series of 37 American patients, neuroborreliosis manifested approximately two years after erythema migrans as subacute encephalopathy; axonal polyradiculoneuropathy with objective sensory and electromyography abnormalities; or leukoencephalitis; or a combination of the three (42). It is important to note that the patients had objective findings and serological evidence of infection.

Late neuroborreliosis treatment: All of the original studies examining treatment of neuroborreliosis that were identified in our search were undertaken in Europe (56, 71–77). While the clinical manifestations of Lyme disease are somewhat different in Europe and North America, presumably the treatment principles are similar. These different studies compared a variety of different drugs, treatment doses and treatment durations. Generally, however, the comparators were intravenous ceftriaxone/cefotaxime, intravenous penicillin or oral doxycycline (200 mg–400 mg daily) for anywhere between 10 days and 3 weeks. Patients had a mix of early and late neurological disease, and follow-up was often for several months after treatment had stopped. The treatment success was comparable for the different treatment arms, but the dose of doxycycline was higher in some of these studies than that used for early Lyme disease. Treatment success was generally higher than 80% but as low as 33% in one group of patients with late Lyme neuroborreliosis who received ceftriaxone for two weeks.

What is the prognosis after delayed treatment?

There are relatively few data examining the effects of delayed treatment. Two retrospective studies showed that patients who had a longer duration of symptoms before treatment were more likely to have persistent subjective musculoskeletal and cognitive symptoms. However, there were no objective physical findings or neurocognitive

testing abnormalities compared with uninfected controls (78, 79). Similar findings were reported in patients involved in studies of early Lyme disease, in which it was found that those who did not receive treatment on initial presentation with facial palsies were more likely to have body pain, physical limitations and a lower physical composite score on the SF36 (36 item Short-Form General Health Survey) compared with controls who received antibiotics at the time of presentation. They were also more likely to have mild neurocognitive symptoms, but their overall "mental composite" score on the SF-36 standardized form was not different from that of the control group (61).

Can patients with Lyme disease have persistent symptoms after treatment?

It is clear that a proportion of patients with confirmed evidence of previous Lyme disease continue to have symptoms after standard antimicrobial treatment. The terms "chronic Lyme disease" and "post-Lyme disease syndrome" have been applied by some clinicians to patients with symptoms persisting more than six months after treatment with the recommended agents. Persistent symptoms include fatigue, generalized musculoskeletal pain and cognitive impairment without objective findings or microbiological evidence of active infection.

Given that some patients have persistent symptoms after treatment of their Lyme disease, the question has been raised of whether there is benefit to prolonged antimicrobial therapy. Four randomized placebo controlled trials, all conducted in the US, have addressed this issue. The larger of the studies (80) included two separate studies (i.e. seropositive and seronegative studies) of patients clinically diagnosed with acute Lyme disease. Both studies enrolled patients who had previously been treated with a recommended antibiotic regimen and reported ongoing symptoms. The subjects were placed into the seropositive or seronegative study according to their serological status at the time of enrollment. There were 129 patients enrolled in the two studies, and participants were randomly assigned to receive either ceftriaxone 2 g daily for 30 days followed by doxycycline for 60 days, or placebo. Outcome measures included cognition, memory, pain and activities of daily living.

There were no differences between the treatment vs placebo groups in outcomes in either the seronegative or seropositive studies, and they were terminated early after an interim analysis by the Data and Safety Monitoring Board, on the basis that the studies would not be able to identify a difference between treatment vs placebo. A sub-study using data from this trial also found no differences between the treatment and control groups in mood and cognitive-related quality of life measures (81).

Krupp and colleagues compared 2 g daily of ceftriaxone for 28 days with placebo in 55 patients whose Lyme disease had been previously treated with a standard regimen (82). While the ceftriaxone group showed improvement in fatigue, there was no difference in cognition on formal testing. Three patients had intravenous line infections and one had anaphylaxis in reaction to ceftriaxone. Finally, 37 adults were randomly assigned to receive 10 weeks of ceftriaxone or placebo after completing treatment for Lyme disease (83). The patients who received ceftriaxone had non-sustained, mild improvements in cognition and a 26.1% adverse event rate versus 7.1% in the placebo group.

On the basis of these findings, the Infectious Diseases Society of America guideline does not recommend additional antimicrobial or prolonged treatment for patients who have completed a standard course of therapy for their Lyme disease (19). Any benefit that might be derived is small and not sustained, and has been associated with an excess risk of adverse events that may be life-threatening.

How should Lyme disease in pregnancy be managed?

While there have been reports of Lyme disease in pregnant women and sporadic case reports of transplacental transmission of *B burgdorferi*, there is not a clear link between fetal infections and adverse outcomes (84, 85). Clinical, serological and epidemiological studies of *Borrelia* infection in pregnancy have failed to demonstrate an association between infection and adverse outcomes (84–86). Because doxycycline is contraindicated in pregnancy, treatment and prophylactic options are different than in the non-pregnant adult.

Some data suggest that a 10 day course of ampicillin may be an effective prophylactic strategy, although the risk of a rash developing in reaction to the beta lactam is greater than the risk of Lyme disease, and therefore the Infectious Diseases Society of America(IDSA) guideline does not recommend prophylaxis in the setting of

pregnancy (18,19,86). If an *Ixodes* tick bites a pregnant woman, she should be monitored for 30 days for signs and symptoms of Lyme disease and treated with amoxicillin or cefuroxime if it develops (19).

Can individuals be re-infected with *Borrelia burgdorferi*?

Relapses are recurrent symptoms that are the result of failure to cure the original infection, whereas re-infection is the recurrence of symptoms as a result of a new exposure to an infected tick, leading to a new infection. Although erythema migrans lesions can relapse if not treated with antibiotics (23), their recurrence after successful treatment is more likely to be re-infection than relapse (87).

Re-infection can occur in as many as 2%–21% of patients living in endemic areas who have had Lyme disease (78, 88–90). On examination, re-infection typically presents with an erythema migrans lesion at a different site than the original lesion more than 1–2.5 years after the original infection and not within 11 months of the first infection (87, 89, 91). In one series, 79% of patients with re-infection presented with erythema migrans at a different site than the previous infection, and 21% presented with a febrile illness with myalgias. Re-infection after late Lyme disease characterized by arthritis or neuroborreliosis is very rare (90, 91).

Laboratory diagnosis of re-infection becomes a challenge, given that serology, including IgM, can remain positive for many years (92). Diagnosis is reliant on the clinical presentation of a new erythema migrans lesion at a different site. Recent data suggest that patients treated for re-infection have excellent outcomes. Patients with re-infection reported less fatigue than both the non-infected control group and patients with their first infection, suggesting that repeated infections present a lower risk of persistent symptoms (91).

Conclusions

Lyme disease is an emerging infection in Canada, and it is important that clinicians be aware of its epidemiology, clinical presentations and management. While sporadic cases of Lyme disease are possible from infected ticks that are imported on migrating birds, individuals living in or traveling to Lyme-endemic regions will be at greatest risk of infection. A thorough travel history is essential in a person presenting with symptoms suggestive of Lyme disease, particularly if symptoms compatible with one of the neurologic syndromes that are more common in Europe are present. It is important to inform the laboratory of a positive travel history, as the serologic testing for North American and European *Borrelia* strains is different.

Given the constantly expanding tick population it can be challenging to clearly define regions constituting the highest risk for Lyme disease, and it is important to remain abreast of local Lyme epidemiology through local public health.

Conflict of interest

The authors have no conflicts of interest to declare.

References

(1) Kuehn, BM. CDC estimates 300000 US cases of Lyme diseaseannually. JAMA. 2013;310:1110

(2) Henry B, Roth D, Reilly R, MacDougall L, Mak S, Li M, et al. How big is the Lyme problem? Using novel methods to estimate the true number of Lyme diseasecases in British Columbia residents from 1997 to 2008. Vector Borne Zoonotic Dis. 2011;11:863-8.

(3) Owens B. Risk of tick-borne infections on the rise. CMAJ. 2013;185:E711.

(4) Ogden NH, Koffi, JK, Pelcat Y, Lindsay LR. Environmental risk from Lyme disease in central and eastern Canada: a summary of recent surveillance information. CCDR. 2014; 40:74-83

(5) Ogden NH, Lindsay LR, Morshed M, Sockett PN, Artsob H. The emergence of Lyme disease in Canada. CMAJ. 2009;180:1221-4.

(6) Lindsay LR, Bernat K, Dibernardo A. Laboratory diagnostics for Lyme disease. CCDR 2014;40:209-217

(7) Huegli D, Moret J, Rais O, Moosmann Y, Erard P, Malinverni R, et al. Prospective study on the incidence of infection by *Borrelia burgdorferi* sensu lato after a tick bite in a highly endemic area of Switzerland. Ticks Tick Borne Dis. 2011;2:129-36.

(8) Costello CM, Steere AC, Pinkerton RE, Feder HM Jr. A prospective study of tick bites in an endemic area for Lyme disease. J Infect Dis. 1989;159:136-9.

(9) Shapiro ED, Gerber MA, Holabird NB, Berg AT, Feder HM Jr, Bell GL, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. N Engl J Med 1992;327:1769-74.

(10) Agre F, Schwartz R. The value of early treatment of tick bites for the prevention of Lyme disease. Am J Dis ChiLyme disease. 1993;147:945–7.

(11) Nadelman RB, Nowakowski J, Fish D, Falco RC, Freeman K, McKenna D, et al., Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an Ixodes scapularis tick bite. N Engl J Med. 2001;345:79-84.

(12) Sood SK, Salzman MB, Johnson BJB, Happ CM, Feig K, Carmody L, et al. Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. J Infect Dis. 1997;175:996–9.

(13) Pal U, Yang X, Chen M, Bockenstedt LK, Anderson JF, Flavell RA et al. OspC facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. J Clin Invest. 2004;113:220-30.

(14) Piesman J, Zeidner NS, Schneider BS. Dynamic changes in *Borrelia burgdorferi* populations in *Ixodes scapularis* (Acari: Ixodidae) during transmission: studies at the mRNA level. Vector Borne Zoonotic Dis. 2003;3:125-32.

(15) Schwan TG, Piesman J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. J Clin Microbiol. 2000;38:382-8.

(16) Radolf JD, Caimano MJ, Stevenson B, Hu LT. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme diseases pirochaetes. Nat Rev Microbiol. 2012;10:87-99.

(17) Warshafsky S, Nowakowski J, Nadelman RB, Kamer RS, Peterson SJ, Wormser GP. Efficacy of antibiotic prophylaxis for prevention of Lyme disease. J Gen Intern Med. 1996;11:329-33.

(18) Warshafsky S, Lee DH, Francois LK, Nowakowski J, Nadelman RB, Wormser GP. Efficacy of antibiotic prophylaxis for the prevention of Lyme disease: an updated systematic review and meta-analysis. J Antimicrob Chemother. 2010;65:1137-44.

(19) Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2006 Nov 1;43:1089-134.

(20) Steere AC, Sikand VK, Schoen RT, Nowakowski J. Asymptomatic infection with *Borrelia burgdorferi*. Clin Infect Dis. 2003;37:528-32.

(21) Petersen LR, Sweeney AH, Checko PJ, Magnarelli LA, Mshar PA, Gunn RA, et al. Epidemiological and clinical features of 1,149 persons with Lyme disease identified by laboratory-based surveillance in Connecticut. Yale J of Biol Med 1989;62:253-62.

(22) Steere AC, Sikand VK. The presenting manifestations of Lyme disease and the outcomes of treatment. N Engl J Med. 2003;348:2472-74.

(23) Steere AC, Bartenhagen NH, Craft JE, Hutchinson GJ, Newman JH, Rahn DW, et al. The early clinical manifestations of Lyme disease. Ann Intern Med. 1983;99:76-82.

(24) Hengge UR, Tannapfel A, Tyring SK, Erbel R, Arendt G, Ruzicka T. Lyme borreliosis. Lancet Infect Dis. 2003;3:489-500.

(25) Nadelman RB, Luger SW, Frank E, Wisniewski M, Collins JJ, Wormser GP. Comparison of cefuroxime axetil and doxycycline in the treatment of early Lyme disease. Ann Intern Med. 1992;117:273-80.

(26) Luger SW, Paparone P, Wormser GP, Nadelman RB, GrunwaLyme diseaset E, Gomez G, et al. Comparison of cefuroxime axetil and doxycycline in treatment of patients with early Lyme disease associated with erythema migrans. Antimicrob Agents Chemother. 1995;39:661-7.

(27) Luft BJ, Dattwyler RJ, Johnson RC, Luger SW, Bosler EM, Rahn DW, et al. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med. 1996;124:785-91.

(28) Wormser GP, Ramanathan R, Nowakowski J, McKenna D, Holmgren D, Visintainer P, et al. Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2003;138:697-704.

(29) Strle F, Maraspin V, Furlan-Lotric S, Cimperman J. Epidemiological study of a cohort of adult patients with Erythema migrans registered in Slovenia in 1993. Eur J Epidemiol. 1996;12:503-7.

(30) Weber K, Wilske B, Preac-Mursic V, Thurmayr R. Azithromycin versus penicillin V for the treatment of early Lyme borreliosis. Infection. 1993;21:367-72.

(31) Barsic B, Maretic T, Majerus L, Strugar J. Comparison of azithromycin and doxycycline in the treatment of erythema migrans. Infection. 2000;28:153-6.

(32) Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. Antibiotic treatment duration and long-term outcomes of patients with early Lyme disease from a Lyme disease-hyperendemic area. Clin Infect Dis. 2010;50:512-20.

(33) Dattwyler RJ, Luft BJ, Kunkel MJ, Finkel MF, Wormser GP, Rush TJ, et al. Ceftriaxone compared with doxycycline for the treatment of acute disseminated Lyme disease. N Engl J Med. 1997; 337:289-94.

(34) Gerber MA, Shapiro ED, Burke GS, Parcells VJ, Bell GL. Lyme disease in children in southeastern Connecticut. Pediatric Lyme disease Study Group. N Engl J Med. 1996;335:1270-4.

(35) Smith RP, Schoen RT, Rahn DW, Sikand VK, Nowakowski J, Parenti DL, et al. Clinical characteristics and treatment outcome of early Lyme disease in patients with microbiologically confirmed erythema migrans. Ann Intern Med. 2002;136:421-8

(36) Cerar D, Cerar T, Ruzić-Sabljić E, Wormser GP, Strle F. Subjective symptoms after treatment of early Lyme disease. Am J Med. 2010;123:79-86

(37) Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED. Long-term outcomes of persons with Lyme disease. JAMA. 2000;283:609-16.

(38) Steere AC. Lyme disease. N Engl J Med. 1989;321:586-96.

(39) Sigal LH. Early disseminated Lyme disease: cardiac manifestations. Am J Med. 1995;98:25S-28S.

(40) Centers for Disease Control and Prevention (CDC). Three sudden cardiac deaths associated with Lyme carditis - United States, November 2012-July 2013. MMWR Morb Mortal Wkly Rep. 2013;62:993-6.

(41) Bacon RM, Kugeler KJ, Mead PS; Centers for Disease Control and Prevention (CDC). Surveillance for Lyme disease--United States, 1992-2006. MMWR Surveill Summ. 2008;57:1-9.

(42) Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. N Engl J Med. 1990;323:1438-44.

(43) Clark JR, Carlson RD, Sasaki CT, Pachner AR, Steere AC. Facial paralysis in Lyme disease. Laryngoscope. 1985;95:1341-5.

(44) Kuiper H, Devriese PP, de Jongh BM, Vos K, Dankert J. Absence of Lyme borreliosis among patients with presumed Bell's palsy. Arch Neurol. 1992;49:940-3.

(45) Halperin JJ, Golightly M. Lyme borreliosis in Bell's palsy. Long Island Neuroborreliosis Collaborative Study Group. Neurology 1992;42:1268-70.

(46) Cook SP, Macartney KK, Rose CD, Hunt PG, Eppes SC, Reilly JS. Lyme disease and seventh nerve paralysis in children. Am J Otolaryngol. 1997;18:320-3.

(47) Nigrovic LE, Thompson AD, Fine AM, Kimia A. Clinical predictors of Lyme disease among children with a peripheral facial palsy at an emergency department in a Lyme disease-endemic area. Pediatrics. 2008;122:e1080-5.

(48) Eppes SC, Nelson DK, Lewis LL, Klein JD. Characterization of Lyme meningitis and comparison with viral meningitis in children. Pediatrics. 1999;103:957-60.

(49) Ljøstad U, Økstad S, Topstad T, Mygland A, Monstad P. Acute peripheral facial palsy in adults. J Neurol. 2005;252:672-6.

(50) Bremell D, Hagberg L. Clinical characteristics and cerebrospinal fluid parameters in patients with peripheral facial palsy caused by Lyme neuroborreliosis compared with facial palsy of unknown origin (Bell's palsy). BMC Infect Dis. 2011;11:215.

(51) Bremell D, Mattsson N, Wallin F, Henriksson J, Wall M, Blennow K, et al. Automated cerebrospinal fluid cell count - New reference ranges and evaluation of its clinical use in central nervous system infections. Clin Biochem. 2014;47:25-30.

(52) Avery RA, Frank G, Glutting JJ, Eppes SC. Prediction of Lyme meningitis in children from a Lyme disease-endemic region: a logistic-regression model using history, physical, and laboratory findings. Pediatrics. 2006;117:e1-7.

(53) Cohn KA, Thompson AD, Shah SS, Hines EM, Lyons TW, Welsh EJ, et al. Validation of a clinical prediction rule to distinguish Lyme meningitis from aseptic meningitis. Pediatrics. 2012;129:e46-53.

(54) Garro AC, Rutman M, Simonsen K, Jaeger JL, Chapin K, Lockhart G. Prospective validation of a clinical prediction model for Lyme meningitis in children. Pediatrics. 2009;123:e829-34

(55) Vázquez M, Sparrow SS, Shapiro ED. Long-term neuropsychologic and health outcomes of children with facial nerve palsy attributable to Lyme disease. Pediatrics. 2003;112:e93-7.

(56) Oksi J, Nikoskelainen J, Hiekkanen H, Lauhio A, Peltomaa M, Pitkäranta A, et al. Duration of antibiotic treatment in disseminated Lyme borreliosis: a double-blind, randomized, placebo-controlled, multicenter clinical study. Eur J Clin Microbiol Infect Dis. 2007;8:571-81.

(57) Pachner AR, Steiner I. Lyme neuroborreliosis: infection, immunity and inflammation. Lancet Neurol. 2007;6:544-552

(58) Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. Ann Intern Med. 1987;107:725-31

(59) Tory HO, Zurakowski D, Sundel RP. Outcomes of chiLyme diseaseren treated for Lyme arthritis: results of a large pediatric cohort. J Rheumatol. 2010;37:1049-55.

(60) Daikh BE, Emerson FE, Smith RP, Lucas FL, McCarthy CA. Lyme arthritis: a comparison of presentation, synovial fluid analysis, and treatment course in children and adults. Arthritis Care Res. 2013;65:1986-90.

(61) Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. Evaluation of study patients with Lyme disease, 10-20-year follow-up. J Infect Dis. 2001;183:453-60.

(62) Thompson A, Mannix R, Bachur R. Acute pediatric monoarticular arthritis: distinguishing Lyme arthritis from other etiologies. Pediatrics. 2009;123:959-65.

(63) Milewski MD, Cruz AI Jr, Miller CP, Peterson AT, Smith BG. Lyme arthritis in children presenting with joint effusions. J Bone Joint Surg Am. 2011;93:252-60.

(64) Deanehan JK, Kimia AA, Tan Tanny SP, Milewski MD, Talusan PG, Smith BG, et al. Distinguishing Lyme from septic knee monoarthritis in Lyme disease-endemic areas. Pediatrics. 2013;131:e695-701.

(65) Steere AC, Levin RE, Molloy PJ, Kalish RA, Abraham JH 3rd, Liu NY, et al.. Treatment of Lyme arthritis. Arthritis Rheum. 1994;37:878-88.

(66) Li X, McHugh GA, Damle N, Sikand VK, Glickstein L, Steere AC. Burden and viability of *Borrelia burgdorferi* in skin and joints of patients with erythema migrans or Lyme arthritis. Arthritis Rheum. 2011;63:2238-47.

(67) Caperton EM, Heim-Duthoy KL, Matzke GR, Peterson PK, Johnson RC. Ceftriaxone therapy of chronic inflammatory arthritis. A double-blind placebo controlled trial. Arch Intern Med. 1990;150:1677-82.

(68) Steere AC, Green J, Schoen RT, Taylor E, Hutchinson GJ, Rahn DW, et al. Successful parenteral penicillin therapy of established Lyme arthritis. N Engl J Med. 1985;312:869-74.

(69) Dattwyler RJ, Wormser GP, Rush TJ, Finkel MF, Schoen RT, GrunwaLyme diseaset E, et al. A comparison of two treatment regimens of ceftriaxone in late Lyme disease. Wien Klin Wochenschr. 2005;117:393-7.

(70) Coyle PK, Schutzer SE. Neurologic aspects of Lyme disease. Med Clin North Am. 2002;86:261-84.

(71) Pfister HW, Preac-Mursic V, Wilske B, Einhäupl K. Cefotaxime vs penicillin G for acute neurologic manifestations in Lyme borreliosis. A prospective randomized study. Arch Neurol. 1989:46:1191-4.

(72) Kohlhepp W, Oschmann P, Mertens H-G. Treatment of Lyme borreliosis. Randomized comparison of doxycycline and penicillin G. J Neurol. 1989;236:464-9.

(73) Pfister H, Preac-Mursic V, Wilske B, Schielke E, Sörgel F, Einhäupl K. Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis. J Infect Dis. 1991:163:311-8.

(74) Karlsson M, Hammers-Berggren S, Lindquist L, Stiernstedt G, Svenungsson B. Comparison of intravenous penicillin G and oral doxycycline for treatment of Lyme neuroborreliosis. Neurology. 1994:44:1203-7.

(75) Roháčová H, Hanči J, Hulinská D, Mailer H, Havlík J. Ceftriaxone in the treatment of Lyme neuroborreliosis. Infection. 1996;24:90-2.

(76) Borg R, Dotevall L, Hagberg L, Maraspin V, Lotric-Furlan S, Cimperman J, et al. Intravenous ceftriaxone compared with oral doxycycline for the treatment of Lyme neuroborreliosis. Scand J Infect Dis. 2005:37:449-54.

(77) Ljøstad U, Skogvoll E, Eikeland R, Midgard R, Skarpaas T, Berg A, et al. Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. Lancet Neurol. 2008:7:690–95.

(78) Shadick NA, Phillips CB, Logigian EL, Steere AC, Kaplan RF, Berardi VP, et al. The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. Ann Intern Med. 1994;121:560-7.

(79) Shadick NA, Phillips CB, Sangha O, Logigian EL, Kaplan RF, Wright EA, et al. Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Ann Intern Med. 1999;131:919-26.

(80) Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med. 2001;345:85-92.

(81) Kaplan RF, Trevino RP, Johnson GM, Levy L, Dornbush R, Hu LT, et al. Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology. 2003;60:1916-22.

(82) Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, et al. Study and treatment of post Lyme disease(STOP-LYME DISEASE): a randomized double masked clinical trial. Neurology. 2003;60:1923-30.

(83) Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology. 2008;70:992-1003.

(84) Walsh CA, Mayer EW, Baxi LV. Lyme disease in pregnancy: case report and review of the literature. Obstet Gynecol Surv. 2007;62:41-50.

(85) Mylonas I. Borreliosis during pregnancy: a risk for the unborn children disease? Vector Borne Zoonotic Dis. 2011;11:891-8.

(86) Smith GN, Gemmill I, Moore KM. Management of tick bites and Lyme disease during pregnancy. J Obstet Gynaecol Can. 2012;34:1087-91.

(87) Nadelman RB, Hanincová K, Mukherjee P, Liveris D, Nowakowski J, McKenna D, et al. Differentiation of reinfection from relapse in recurrent Lyme disease. N Engl J Med. 2012;367:1883-90.

(88) Nowakowski J, Nadelman RB, Sell R, McKenna D, Cavaliere LF, Holmgren D, et al. Long-term follow-up of patients with culture-confirmed Lyme disease. Am J Med. 2003;115:91-6.

(89) Krause PJ, Foley DT, Burke GS, Christianson D, Closter L, Spielman A; Reinfection and relapse in early Lyme disease. Tick-Borne Disease Study Group. Am J Trop Med Hyg. 2006;75:1090-4.

(90) Nadelman RB, Wormser GP. Reinfection in patients with Lyme disease. Clin Infect Dis. 2007;45:1032-8.

(91) Jares TM, Mathiason MA, Kowalski TJ. Functional outcomes in patients with *Borrelia burgdorferi* reinfection. Ticks and tick-borne dis. 2014;5:58-62.

(92) Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10–20 years after active Lyme disease. Clin Infect Dis 2001;33:780–5.

Laboratory diagnostics for Lyme disease

Lindsay LR[1*], Bernat K[1] and Dibernardo A[1]

[1] National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba
* Corresponding author: robbin.lindsay@phac-aspc.gc.ca

Abstract

Background: Lyme disease is on the rise in Canada. It is a notifiable disease, and when infection is disseminated, serological testing provides supplemental evidence to confirm a case.

Objective: To describe the current diagnostic tests for Lyme disease, review the recommended approach to laboratory testing for Lyme disease and identify future research priorities for Lyme disease laboratory diagnostics in Canada.

Methods: A review of the literature was carried out. We then summarized parameters to consider before Lyme disease testing is conducted, described the current best practice to use a two-tiered diagnostic algorithm for the laboratory confirmation of disseminated Lyme disease, and analyzed the advantages and disadvantages of the supplemental tests for Lyme disease.

Results: Diagnostic testing is indicated in people who have symptoms of disseminated disease and a history of exposure to vector ticks. To maximize sensitivity and specificity, a two-tiered serological approach is recommended, consisting of an enzyme immunoassay (EIA) screening test followed by confirmation with Western blot (WB) testing. A number of other diagnostic tests are available; however, these are largely for research purposes.

Conclusion: Two-tiered serology is currently the best approach available to assist doctors when they are making a diagnosis of disseminated Lyme disease. The Public Health Agency of Canada (the Agency) will seek to improve on this approach through standardization of the Lyme disease diagnostics used across laboratories in Canada, evaluation of test performance characteristics of current and new diagnostic platforms and development of a process to secure robust serum panels to assist in the development and evaluation of new diagnostic tests for Lyme disease.

Introduction

Lyme disease (LD) is a tick-borne infection caused primarily by three species of spirochetes in the *Borrelia burgdorferi* sensu lato genogroup: *B. burgdorferi* sensu stricto (in North America and Western Europe), *B. afzelii* (in Western Europe, central Europe and Russia) and *B. garinii* (primarily in Europe, Russia and northern Asia) (1). The symptoms of Lyme disease occur in stages and involve a variety of tissues and organs, including the skin, joints, heart and nervous system (2). There has been a steady increase in the incidence of Lyme disease in parts of central and eastern Canada (3-5) due to the recent range expansion of the primary tick vector, *Ixodes scapularis* (6).

Lyme disease has been a nationally notifiable disease since 2009 (7). The objective of this article is to describe the current diagnostic tests for Lyme disease, including a review of the recommended approaches to laboratory testing, and identify future research priorities for Lyme disease diagnostics in Canada.

Methods

An extensive review of peer-reviewed literature was carried out. We then summarized the key parameters to consider before Lyme disease testing is conducted, described the current best practice of a two-tiered serological algorithm for the laboratory confirmation of disseminated Lyme disease and explored the advantages

and disadvantages of the supplemental tests. We also outlined future research plans to be undertaken by the Agency's National Microbiology Laboratory.

Results

Considerations prior to testing

Early localized Lyme disease does not require diagnostic testing before antibiotic therapy is started. A presumptive diagnosis can be made on the basis of the clinical presentation and a credible history of exposure to infected blacklegged ticks (8). Typically, diagnostic testing is appropriate for people with a history of tick exposure and symptoms of disseminated Lyme disease infection, since test sensitivity improves as the bacteria affect tissue systems other than the skin (8,9). Testing, however, should be limited to those with objective signs of infection (10,11).

The following information is required prior to testing:

- **Detailed travel history and date of onset of symptoms** – This information should be included on the laboratory requisition, as it helps the diagnostic laboratory apply the most appropriate test platform. For example, there are different tests to identify Lyme disease acquired in Europe/Asia versus North America (12), and different tests are used for early infections versus infections that may have been present for some time (13).
- **A history of antibiotic treatment** – This can dampen the immune response to infection and may complicate the interpretation of serological tests (14).
- **Other infections or pre-existing conditions** – Infection with other related pathogens (e.g. syphilis) and autoimmune disorders may cause false-positive results (15).
- **Prior history of laboratory-confirmed Lyme disease**– This is important, as there is no pattern of serological response that can differentiate re-infection from an initial infection with *B. burgdorferi* (16).

Testing for Lyme disease

Although there are several testing strategies that can assist in making a diagnosis of Lyme disease (**Table 1**), serology is currently the only standardized laboratory testing available. The following describes the different test platforms used for the laboratory diagnosis of Lyme disease. The advantages and limitations of each are presented in **Table 2**.

Table 1: Laboratory testing approaches for Lyme disease (9)

Stage of infection	Recommended testing strategy*	Specimen type
Erythema migrans, acute phase (seasonal occurrence and exposure in an endemic area**)	Clinical diagnosis and empirical treatment	None
Erythema migrans, acute phase (out of season or no known exposure in an endemic area)	2-tiered serology† – repeat EIA in four weeks if negative; treatment at physician's discretion NAAT, isolation	Serum Biopsy, plasma
Characteristic neurological, cardiac or joint involvement	2-tiered serology† NAAT	Serum Synovial or cerebrospinal fluid
Persistent symptoms following recommended treatment	None	None

* Tests, such as nucleic acid amplification test (NAAT) or bacterial isolation, are not frequently requested or performed.
** Endemic areas are localities where blacklegged ticks are established and *B. burgdorferi* cycles of transmission are maintained.
† Enzyme immunoassay (EIA) followed, where appropriate, by confirmatory Western blots using diagnostic kits licensed in Canada.

Serology testing

The Canadian Public Health Laboratory Network recommends a two-tiered approach to Lyme disease testing, consisting of a sensitive enzyme immunoassay (EIA) followed, if positive or equivocal, by a specific Western blot test (9). The rationale for this approach is that the overall sensitivity and specificity are maximized when these tests are performed in sequence.

The immune response to *B. burgdorferi* infection begins with the appearance of IgM antibodies, usually within two weeks of a tick bite (17). These antibodies may persist for months or even years despite effective antimicrobial therapy (18). Following that IgM response, IgG antibodies develop in most patients, typically after one month of infection (9).

Serology provides a snapshot of the immune status of the patient at the time of the specimen collection. For instance, if Lyme disease is suspected on the basis of symptoms but early serological testing is negative; follow-up testing on a convalescent sample is recommended (9). The two most commonly performed serological tests are detailed below.

Enzyme immunoassay (EIA)

An enzyme immunoassay is used as a screening test to detect IgM and/or IgG antibodies in serum that are directed against the bacterium that causes LYME DISEASE. Commercial kits, such as an enzyme-linked immunosorbent assay, rely on the use of whole-cell preparations of *B. burgdorferi* (1) and/or recombinant antigens (19) (e.g. C6 peptide). The format of the assay allows the simultaneous screening of a relatively large number of samples. While most enzyme immunoassays are highly sensitive, they may lack specificity (i.e. false positives can occur as a result of other medical conditions).

Western blot (WB)

The Western blot test is used as the corroborative test, and it has greater specificity than the enzyme immunoassay (11, 20). It detects antibodies in serum that are directed against electrophoretically separated antigen extracts and recombinant antigens native to *B. burgdorferi* (21). Commercial kits are used to test for antibodies to individual genospecies of *Borrelia* (12) and to differentiate IgM from IgG antibodies. A positive Western blot result is required to confirm exposure to *B. burgdorferi* (22), and seroconversion from IgM to IgG Western blot antibodies provides definitive evidence of a recent infection (9).

Table 2: Laboratory tests for Lyme disease and their advantages and limitations

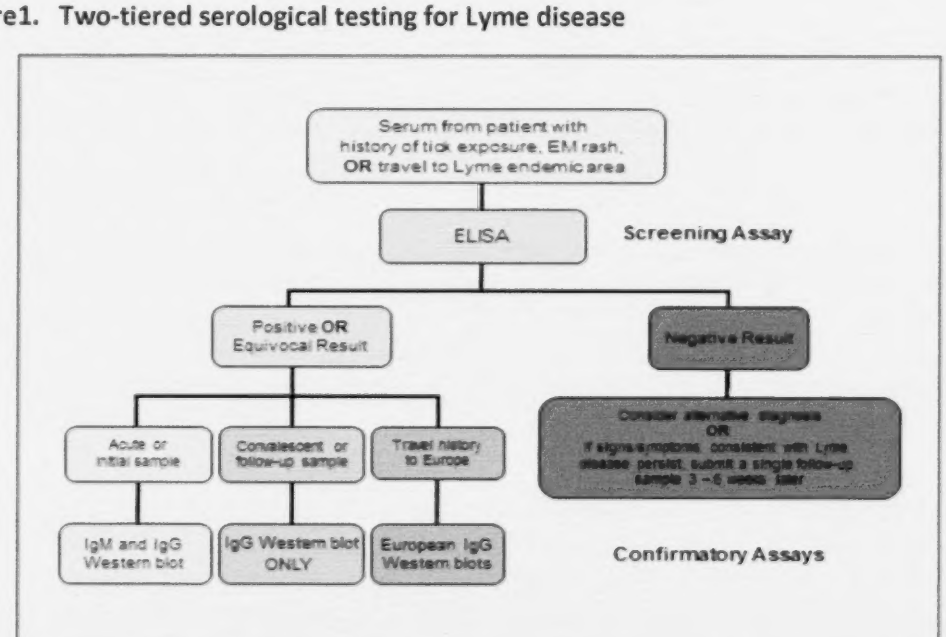
Test	Advantages	Limitations
Enzyme immunoassay	• High sample throughput and relatively easy to perform. • Generates objective numerical values compared with other subjective measures (e.g. immunofluorescent assays).	• Lower sensitivity in early stage Lyme disease. • There is variation in the sensitivities and specificities of the different commercial kits available in Canada. • Antibodies in the serum of patients with autoimmune disorders, Epstein-Barr virus infection, bacterial endocarditis, syphilis, other spirochetal infections, anaplasmosis or *Helicobacter pylori* infection may cause false-positive results. • Some tests cannot discriminate between antibodies produced against North American versus European/Asian genospecies of *Borrelia*. • Genotype of *B. burgdorferi* may reduce sensitivity in early Lyme disease. • Cannot differentiate a previous infection from a re-infection with *B. burgdorferi*.

Test	Advantages	Limitations
Western blot	• High specificity such that these tests can be used to rule out other etiologic agents. • Able to determine reactive immunoglobulin classes (IgG vs. IgM) and help differentiate early from longer-standing infections.	• Interpretation of results is subjective (e.g. scoring band position and intensity) for Western blot assays that do not use an automated reader. • Significant cross-reactivity occurs among European genospecies. • LgM antibodies are inherently cross-reactive, which may lead to false-positive results. • False-negative IgG Western blot results may occur early in the course of infection or as a result of antibiotic treatment.
SUPPLEMENTAL LABORATORY TESTS (Not routinely performed)		
Bacterial isolation	• Highly specific and could be useful for determining infecting genotypes of *B. burgdorferi*.	Collection of specimens can be invasive. Relatively low sensitivity, expensive, labour-intensive and long incubation period required for results.
Nucleic acid amplification testing (NAT)	• Able to detect *B. burgdorferi* DNA after antibiotic treatment has started, therefore able to distinguish an ongoing infection from persistent symptoms due to an immunologic mechanism. • *Borrelia* DNA can be detected in EM lesions before the appearance of serum antibodies and without the delay associated with bacterial isolation.	• Poor sensitivity due to low bacterial load in some clinical samples. • Lack of standardization with respect to target genes.

Two-tiered algorithm for the laboratory diagnosis of Lyme disease

The two-tiered approach to testing is illustrated in **Figure 1**. The first tier involves the use of an EIA. If this EIA test is negative, WB testing is not indicated. If symptoms persist, the EIA test can be repeated on a convalescent sample collected 3–6 weeks later. If the EIA is positive or equivocal, the second tier or corroborative Western blot assay is performed. In early infections (i.e. symptoms for less than six weeks), both the IgM and IgG Western blot tests are performed; however, if the patient has had symptoms for more than six weeks, only the IgG Western blot assay is performed.

Figure1. Two-tiered serological testing for Lyme disease


Serum from patient with history of tick exposure, EM rash, OR travel to Lyme endemic area
ELISA
Screening Assay
Positive OR Equivocal Result
Negative Result
Acute or initial sample
Convalescent or follow-up sample
Travel history to Europe
IgM and IgG Western blot
IgG Western blot ONLY
European IgG Western blots
Confirmatory Assays

The final result of serological testing is considered positive only when the EIA is reactive (positive or equivocal) and the WB is also positive (**Table 3**). This two-tiered system maximizes the sensitivity and specificity of the assays and increases the likelihood of observing a seroconversion (from IgM to IgG) that is evident in most *bona fide B. burgdorferi* infections (1, 17, 21, 23).

Table 3: Interpretation of Western blot results (in conjunction with an equivocal or positive EIA)

Western blot result	Interpretation
Both IgM and IgG Western blots negative	Result not consistent with a *B. burgdorferi* infection; however, if symptoms persist submit a follow-up sample 3–6 weeks later.
Only IgM Western blot positive*	Potentially a false-positive result if this is NOT an acute case (i.e. < 6 weeks post onset of symptoms).
Only IgG Western blot positive**	Result consistent with an infection with *B. burgdorferi* of greater than 4 weeks' duration.
Both IgM and IgG Western blots positive	Result indicates recent or previous infection with *B. burgdorferi*.

* IgM positive WB – 2 of 3 significant bands present.
** IgG positive WB – 5 of 10 significant bands present (22).

Supplemental laboratory tests to detect *B. burgdorferi*

Bacterial isolation

The recovery of viable *B. burgdorferi* from clinical specimens is accomplished by incubating the sample in specialized culture medium. Although this test remains the "gold standard" for diagnosis of Lyme disease(7), the procedure is expensive to perform, lacks clinical sensitivity and is prone to contamination (24). The greatest practical limitation is that cultures can require up to eight weeks of incubation because of the small number of viable organisms present in many specimen types (17). These factors reduce the clinical applications of this test and restrict its use to research studies (9).

Nucleic acid amplification testing (NAAT)

Nucleic acid amplification testing (NAAT) has been used to decrease turnaround times for Lyme disease diagnostic results (25, 26). Several formats of polymerase chain reaction (PCR) testing (i.e. nested, real-time or quantitative) are used to amplify a variety of *Borrelia*-specific genetic targets in clinical specimens (27). Positive results are most frequently seen in the early phase of the disease (28). The sensitivity of polymerase chain reaction on cerebrospinal fluid (CSF) is low or variable and therefore of limited usefulness in evaluating patients with neurological signs (1, 21). Although these assays can identify an infection sooner than serological testing (25, 27), their use is restricted to research studies at the present time (8, 9).

Challenges associated with diagnostic testing for Lyme disease

Physicians and laboratory scientists have concerns regarding results reported by some private laboratories that are inconsistent with results obtained by Canadian public health laboratories. A number of private laboratories may not be using sufficiently validated tests or interpretation criteria. The use of assays that do not have adequately established accuracy and have not been sufficiently validated may result in the reporting of false-positive results.

Some of these tests include capture assays for antigens in urine, immunofluorescence staining, cell sorting of cell wall-deficient or cystic forms of *B. burgdorferi*, lymphocyte transformation tests (29) and a new culture method for serum (30).

Currently, not following the two-tiered algorithm (e.g. by performing a Western blot alone or after the EIA is negative) can increase the frequency of false-positive results. This in turn could lead to possible misdiagnosis and unnecessary treatment (1). Clinicians should have an understanding of the current common misconceptions

about LYME DISEASE(31) and know the best laboratory practices to diagnose it (1); this would facilitate informed discussions with patients who have questions and concerns.

Future developments

As mentioned, there are a wide variety of diagnostic tests available to assist in the diagnosis of Lyme disease (21, 32), and considerable debate exists concerning the accuracy and reliability of some of these tests (32–34). At this time there is no single laboratory test that is 100% sensitive and specific for the confirmation of Lyme disease. This is further complicated by the fact that not all individuals who are infected with *B. burgdorferi* present in the same way. Improvements in diagnostic test platforms are a priority. The search for "biomarkers" of Lyme disease that do not rely on serological response is ongoing (35). Variations of the two-tiered approach, which typically reduce or eliminate the use of WBs, are being evaluated (36–39) and may help simplify test interpretation and improve sensitivity in early Lyme disease.

One of the biggest challenges to evaluating new diagnostic approaches (40) in Canada is the lack of serum panels or a collection of well-characterized samples from individuals with confirmed *B. burgdorferi* infection. It is also imperative that we gain a full understanding of the genotypes of *B. burgdorferi* that are infecting Canadians (41) and establish whether the diagnostic tests currently in use can detect all of them with comparable sensitivity.

The Agency's National Microbiology Laboratory plans to work with diagnostic laboratories across Canada to review current Lyme disease diagnostic practices and quality assurance systems, and to evaluate the need for enhanced internal and external proficiency testing. In the longer term, the Agency also plans to 1) review and update the existing laboratory guidelines for Lyme disease, 2) determine and compare test performance characteristics of all EIA and Western blot platforms currently in use in Canada, 3) evaluate "new" diagnostic platforms on a high priority and ongoing basis and 4) initiate the process of development of a robust serum panel for use in evaluation of new assays (42). This work will advance quality diagnostic testing for Lyme disease in Canada.

Conclusion

The incidence of Lyme disease is increasing in Canada. In persons with erythema migrans rash and a reliable history of exposure to blacklegged ticks, testing is not required and treatment can be started empirically. The clinical assessment of patients in the disseminated phases of Lyme disease can be further supported by laboratory testing.

At this time, there is no perfect laboratory test for Lyme disease; however, the two-tiered serological approach provides the most sensitive and specific results to date. Improper use of serologic tests or the use of diagnostic tests or interpretative criteria that have not been fully validated may lead to misdiagnosis and unnecessary antibiotic treatment. Future efforts will focus on standardization of testing, and the development and evaluation of new diagnostic approaches to optimize the detection of Lyme disease.

Acknowledgements

We would like to thank our colleagues in the provincial diagnostic laboratories and hospital laboratories across Canada who provided important and useful feedback on the laboratory diagnostic testing for Lyme disease that is performed at the National Microbiology Laboratory.

Conflict of interest

The authors have no conflicts of interest to declare.

References

(1) Johnson BJB, Aguero-RosenfeLyme diseaseME, Wilske B. Sood SK, editor. Lyme borreliosis in Europe and North America: epidemiology and clinical practice. [Internet]. Hoboken, NJ: John Wiley & Sons, Inc.; 2011Serodiagnosis of Lyme borreliosis ; p. 185-212.cited 28 November 2013]. DOI: 10.1002/9780470933961.ch10

(2) Hatchette TF, Davis I, Johnston BL. Lyme disease: clinical diagnosis and treatment. CCDR. 2014;40-11:194-208.

(3) Ogden NH, Lindsay LR, Morshed M, Sockett PN, Artsob H. The emergence of Lyme disease in Canada. CMAJ [Internet]. 2009 [cited 28 November 2013];180(12):1221-4.

(4) Ogden NH, Artsob H, Lindsay LR, Sockett PN. Lyme disease: A zoonotic disease of increasing importance to Canadians. Can Fam Phys [Internet]. 2008;54(10):1381-4.

(5) Ogden NH, Lindsay LR, Morshed M, Sockett PN, Artsob H. The rising challenge of Lyme borreliosis in Canada. CCDR [Internet]. 2008;34(1):1-19.

(6) Ogden NH, Koffi JK, Pelcat Y, Lindsay LR. Environmental risk from Lyme disease in centeral and eastern Canada: a summary of recent surveillance information. CCDR. 2014;40-5:74-82.

(7) Case definitions for communicable diseases under national surveillance - 2009. CCDR [Internet]. 2009 November 2009;35(Suppl 2):1-123.

(8) Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, Krause PJ, Bakken JS, Strle F, Stanek G, Bockenstedt L, Fish D, Dumler JS, Nadelman RB. The clinical assessments treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis [Internet]. 2006;43(9):1089-134.

(9) Canadian Public Health Laboratory Network. The laboratory diagnosis of Lyme borreliosis: Guidelines from the Canadian Public Health Laboratory Network. Can J Infect Dis Med Microbiol [Internet]. 2007 Mar;18(2):145-8.

(10) Seltzer EG, Shapiro ED. Misdiagnosis of Lyme disease: When not to order serologic tests. Pediatr Infect Dis J [Internet]. 1996;15(9):762-3.

(11) Tugwell P, Dennis DT, Weinstein A, Wells G, Shea B, Nichol G, Hayward R, Lightfoot R, Baker P, Steere AC. Laboratory evaluation in the diagnosis of Lyme disease. Ann Intern Med [Internet]. 1997 Dec 15;127(12):1109-23.

(12) Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. Clin Infect Dis [Internet]. 2013 Aug;57(3):333-40.

(13) Schoen RT. Better laboratory testing for Lyme disease: No more Western blot. Clin Infect Dis [Internet]. 2013 [cited 27 November 2013];57(3):341-3.

(14) Aguero-RosenfeLyme diseaseME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J Clin Microbiol [Internet]. 1996;34(1):1-9.

(15) Johnson BJ, Robbins KE, Bailey RE, Cao BL, Sviat SL, Craven RB, Mayer LW, Dennis DT. Serodiagnosis of Lyme disease: accuracy of a two-step approach using a flagella-based ELISA and immunoblotting. J Infect Dis [Internet]. 1996 Aug;174(2):346-53.

(16) Nadelman RB, Wormser GP. Reinfection in patients with Lyme disease. Clin Infect Dis [Internet]. 2007 Oct 15;45(8):1032-8.

(17) Aguero-RosenfeLyme diseaseME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev [Internet]. 2005 Jul;18(3):484-509.

(18) Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease. Clin Infect Dis [Internet]. 2001;33(6):780-5.

(19) Liang FT, Alvarez AL, Gu Y, Nowling JM, Ramamoorthy R, Philipp MT. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of Borrelia burgdorferi. J Immunol [Internet]. 1999;163(10):5566-73.

(20) Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis [Internet]. 1993 Feb;167(2):392-400.

(21) Aguero-RosenfeLyme diseaseME. Lyme disease: laboratory issues. Infect Dis Clin North Am [Internet]. 2008 [cited 27 November 2013];22(2):301-13.

(22) Centers for Disease Control and Prevention (CDC). Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme disease. MMWR Morb Mortal Wkly Rep [Internet]. 1995 Aug 11;44(31):590-1.

(23) Craven RB, Quan TJ, Bailey RE, Dattwyler R, Ryan RW, Sigal LH, Steere AC, Sullivan B, Johnson BJ, Dennis DT, Gubler DJ. Improved serodiagnostic testing for Lyme disease: results of a multicenter serologic evaluation. Emerg Infect Dis [Internet]. 1996 Apr-Jun;2(2):136-40.

(24) Nowakowski J, Schwartz I, Liveris D, Wang G, Aguero-RosenfeLyme diseaseME, Girao G, McKenna D, Nadelman RB, Cavaliere LF, Wormser GP, Lyme diseaseStudy Group. Laboratory diagnostic techniques for patients with early Lyme diseaseassociated with erythema migrans: a comparison of different techniques. Clin Infect Dis [Internet]. 2001 Dec 15;33(12):2023-7.

(25) Wormser GP, Wang G. Sood SK, editor. Lyme borreliosis in Europe and North America : epidemiology and clinical practice. [Internet]. Hoboken, N.J.: John Wiley & Sons, Inc.; 2011The role of culture and nucleic acid amplification in diagnosis of Lyme borreliosis ; p. 159-83.. DOI: 10.1002/9780470933961.ch9

(26) Dumler JS. Molecular diagnosis of Lyme disease: review and meta-analysis. Mol Diagn [Internet]. 2001 Mar;6(1):1-11.

(27) Eshoo MW, Schutzer SE, Crowder CD, Carolan HE, Ecker DJ. Achieving molecular diagnostics for Lyme disease. Expert Rev Mol Diagn [Internet]. 2013 Nov;13(8):875-83.

(28) Liveris D, Schwartz I, McKenna D, Nowakowski J, Nadelman R, Demarco J, Iyer R, Bittker S, Cooper D, Holmgren D, Wormser GP. Comparison of five diagnostic modalities for direct detection of Borrelia burgdorferi in patients with early Lyme disease. Diagn Microbiol Infect Dis [Internet]. 2012 Jul;73(3):243-5.

(29) Centers for Disease Control and Prevention (CDC). Caution regarding testing for Lyme disease. Morb Mortal Wkly Rep [Internet]. 2005;54(5):125.

(30) Johnson BJ, Pilgard MA, Russell TM. Assessment of New Culture Method to Detect Borrelia species in Serum of Lyme diseasePatients. J Clin Microbiol [Internet]. 2013 Aug 14

(31) Halperin JJ, Baker P, Wormser GP. Common misconceptions about Lyme disease. Am J Med [Internet]. 2013 [cited 28 November 2013];126(3):264.e1,264.e7.

(32) Johnson BJB. Halperin JJ, editor. Lyme disease: An Evidence-based Approach. Cambridge, MA, USA: CABI Publishing; 2011 Laboratory diagnostic testing for *Borrelia burgdorferi* infection ; p. 73-88.;

(33) Bowie WR. Guidelines for the management of Lyme disease: The controversy and the quandary. Drugs [Internet]. 2007;67(18):2661-6.

(34) Halperin JJ, Baker P, Wormser GP. Halperin JJ, editor. Lyme disease: An Evidence-based Approach. Cambridge, MA, USA: CABI Publishing; 2011Lyme disease: the Great Controversy ; p. 259-70.

(35) Cerar T, Ogrinc K, Lotric-Furlan S, Kobal J, Levicnik-Stezinar S, Strle F, Ruzic-Sabljic E. Diagnostic value of cytokines and chemokines in Lyme neuroborreliosis. Clin Vaccine Immunol [Internet]. 2013 Oct;20(10):1578-84.

(36) Porwancher RB, Hagerty CG, Fan J, Landsberg L, Johnson BJB, Kopnitsky M, Steere AC, Kulas K, Wong SJ. Multiplex immunoassay for Lyme disease using VlsE1-IgG and pepC10-IgM antibodies: Improving test performance through bioinformatics. Clin Vaccine Immunol [Internet]. 2011;18(5):851-9.

(37) Wormser GP, Schriefer M, Aguero-Rosenfe ME, Levin A, Steere AC, Nadelman RB, Nowakowski J, Marques A, Johnson BJB, Dumler JS. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. Diagn Microbiol Infect Dis [Internet]. 2013;75(1):9-15.

(38) Branda JA, Linskey K, Kim YA, Steere AC, Ferraro MJ. Two-tiered antibody testing for Lyme diseasewith use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a vlse c6 peptide enzyme immunoassay. Clin Infect Dis [Internet]. 2011;53(6):541-7.

(39) Branda JA, Aguero-RosenfeLyme diseaseME, Ferraro MJ, Johnson BJB, Wormser GP, Steere AC. 2-Tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test. Clin Infect Dis [Internet]. 2010;50(1):20-6.

(40) Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for Lyme disease. Clin Infect Dis [Internet]. 2008 [cited 27 November 2013];47(2):188-95.

(41) Ogden NH, Margos G, Aanensen DM, Drebot MA, Feil EJ, Hanincová K, Schwartz I, Tyler S, Lindsay LR. Investigation of genotypes of *Borrelia burgdorferi* in *Ixodes scapularis* ticks collected during surveillance in Canada. Appl Environ Microbiol [Internet]. 2011;77(10):3244-54.

(42) Aguero-RosenfeLyme diseaseME. Committee on Lyme diseaseand Other Tick-Borne Diseases: The State of the Science, Institute of Medicine., editors. Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases [electronic resource] : the short-term and long-term outcomes : workshop report. [Internet]. Washington, DC: National Academies Press; 2011Diagnostics for Lyme disease: Knowledge gaps and needs ; p. 125-30.